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RESPONSES OF CIGARETTE BEETLE LARVAE TO TEMPERATURE AND HUMIDITY

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RESPONSES OF CIGARETTE BEETLE LARVAE TO TEMPERATURE AND HUMIDITY

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INTRODUCTION

The cigarette beetle, *Lasioderma serricorne* F., usually overwinters in the Southeastern United States as larvae (13).⁶ Reed and Vinzant (12) suggested that severe winters in North Carolina, Tennessee, and States farther north materially reduce overwintering larval populations by killing off early instars. Another factor that may help reduce larval infestations is the little understood phenomenon of larval cigarette beetle "emigration," defined in this paper as the movement of large numbers of larvae from stored tobacco. Workers in tobacco storages during the fall season of some years have observed large-scale emigrations of late instar larvae from tobacco stored in unheated warehouses. Although

only general observations of the environmental conditions prevailing during the periods of movement are available, a distinct pattern of phenological events can be described.

The emigrations, seldom over one each year, occur only during the fall. Most are observed from September 15 to October 15. Before the larval emigration, temperatures within the periphery of tobacco stored in hogsheads or bales are such, 68° F or less, that the larvae are relatively inactive. Sudden warm weather creates a condition in the warehouse where air temperature is somewhat higher than tobacco temperature. As the tobacco warms, temperature and humidity gradients form in the tobacco perimeter. Then at some point during the warming cycle, cigarette beetle larvae crawl from the tobacco and fall to the floor. The floor of the warehouse is cool, and larval movement is restricted to the extent that they are unable to find a suitable overwintering habitat. They usually die later from dehydration and cold.

It is possible that larval emigration could be used to control the cigarette beetle in tobacco storages if the emigration could be induced. First, however, the mechanism of the emigration must be understood. In this study we investigated the effect of humidity (section 1) and temperature (section 2) on larval movement under laboratory conditions. Then, we applied our laboratory observations to a study of larval emigration under actual warehouse conditions (section 3).

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SECTION 1.—RESPONSE OF LARVAE TO HUMIDITY GRADIENTS

No information is available about the effects of humidity gradients on larval cigarette beetle movement. Apart from the study on water vapor sorption from the atmosphere by Knülle and Spadafora (9), nothing is known about the water balance of the larvae. In the tests described in this section, the preference of larvae for various relative humidities was investigated by observing their movement in humidity gradient chambers.

Materials and Methods

All experiments were initiated with normal larvae taken from stock cultures reared on cornmeal and yeast in total darkness at 77° F and 60% relative humidity. An aspirator was used to clean the larvae before they were placed, without food, in a humidity gradient chamber. Each experiment was replicated four times. Two types of humidity gradient chambers were employed. One was circular (8); the other was rectangular with rounded corners (14). The circular chamber presented the larvae with two alternative humidities; the rectangular chamber, with two to six humidities in descending order.

The desired relative humidities were established with saturated salt solutions and an excess of solids (8, 14). Temperature was controlled with incubators. All experiments were conducted in the dark at 77° F. Larvae were exposed to light only when distribution counts were recorded. Exposure time did not exceed 20 seconds per count. The chambers were rotated 180° at various times throughout experiments.

In most experiments, 20 and 40 larvae per replication were exposed in the circular and rectangular chambers, respectively. Random distribution was determined by position records in chambers with uniform conditions throughout the test area. The "preference indices" calculated by determining the mean number of larvae per recording on each side of the chamber were compared with random distribution counts for significant differences by the *t*-test. A detailed description of methods and procedures employed with the circular chamber is given by Knülle (8).

The first experiment was made to determine the response of larvae presented with the choice between two different humidities. Only larvae that had molted to the third instar within the past 24 to 48 hours were used. Position records

were made at 10-minute intervals for the first 2 hours and thereafter at 30-minute intervals for an additional 6 hours. The alternative humidities were 73% and 43%. In addition, second, third, and fourth instar larvae were also presented with a choice between the following pairs of humidities: 93% and 85%, 93% and 73%, 73% and 53%, 73% and 33%, and 33% and 13%.

To approximate the response threshold—that is, the point at which the difference between two humidities is small enough that the larvae can no longer discriminate between them—groups of early third and fourth instar larvae were exposed to alternative relative humidities of 65% and 60%, 60% and 55%, and 60% and 58%. The range of 55% and 65% was selected for this series of experiments to approximate warehouse humidities during larval emigration.

In another series of experiments third instar larvae were exposed to six relative humidities arranged in descending order. The movement of the larvae was recorded with the aid of time-lapse photography during the initial 2 hours of exposure.

The effect of preconditioning in low humidity on larval response was investigated by holding larvae at 33% relative humidity for 3 days without food. Thirty-three percent was selected as the dehydration humidity because it is known to be below the humidity at which sorption of water vapor from the atmosphere by larvae is possible (8). The larvae were then presented with alternative humidities of 93% and 85%, 93% and 73%, 73% and 53%, 73% and 33%, and 33% and 13%.

Results and Discussion

The response of third instars to the alternative humidities of 73% and 43% was readily determined. Within the first 10 minutes the larvae showed a strong preference for the drier of the two humidities. There was no appreciable change in the response throughout the 8-hour test. Compared with random distribution in the control (73% r.h. vs. 73% r.h.), differences were significant at the 0.01 level. Likewise, the initial preference of second, third, and fourth instar larvae for the drier humidity of 93% and 85%, 93% and 73%, 73% and 53%, 73% and 33%, and 33% and 13% was obvious.

There was a tendency for the response of some groups of larvae to weaken after the initial 2 to 3 hours of exposure. Often these groups were sluggish, an indication that they were approaching the 12- to 24-hour quiescent period just before molting. It is possible that the attraction to drier conditions is temporarily weakened or lost just before molting as a preventive measure against excessive desiccation. Further exploration into the question, however, is needed.

Third and fourth instar larvae presented with a choice between 65% and 60% and between 60% and 55% relative humidity showed a strong preference for the drier humidity of each pair. Presented with the alternatives of 60% and 58%, larvae also showed a preference for the drier humidity, but the intensity of the response had diminished. These results indicate that the

response threshold is probably in the neighborhood of 1% to 2% relative humidity, at least in the range of humidity studied.

Time-lapse photography of third instar larvae exposed to six relative humidities in descending order showed a definite avoidance of humidities above 43% during the first 2 hours of exposure. Exposures of 1 to 4 days substantiated this observation.

The response of larvae preconditioned by holding in 33% relative humidity for 3 days to five pairs of humidities was similar to that observed for normal, unconditioned, third instar larvae. The groups of larvae that remained active throughout the 8-hour test always displayed an initial preference for the lower humidity of each pair.

SECTION 2.—RESPONSE OF LARVAE TO TEMPERATURE GRADIENTS

The lethal effect of low temperatures on cigarette beetle larvae has received considerable attention (6, 12, 13, 15). However, little is known about the effects of temperature gradients on larval cigarette beetle movement except that activity ceases whenever the temperature falls between 60° and 67° F and that larvae become inactive below about 50° F (Runner, 13). Lefkovich (10) studied larval cigarette beetle movement in a plane. He found that third and fourth instar larvae were rather active under test conditions of 86° F and 60% relative humidity. Distances traveled in 24 hours were often greater than 4 inches. He observed that the rate of movement increased with increased density of larvae. Amos (1, 2) and coworkers (3-5) and Yinon and Shulov (16, 17) have reported on the distribution of several stored-product insect species other than cigarette beetles in relation to temperature and humidity gradients. This part of our study was concerned with the response of third and fourth instar larvae to temperature. Larval densities in preferred temperature zones were also recorded.

Materials and Methods

The two temperature gradient chambers (fig. 1) used to observe larval cigarette beetle movement were a modification of one used by Fletcher and others (7). An iron bar, 54 inches long by 2 to 3 inches wide by 0.25 inch thick, was tapered so that the bar width at the smaller end was 2

inches. The wide end of the bar was then inserted between the two prongs (18 inches long) of an immersion heater. The heating elements were clamped on the bar with two sets of angle irons bolted together over and around the ends of the elements. The heater provided an even distribution of heat on the upper and lower surfaces of the bar. The heating element, powered by a 60-cycle, a.-c., 120/140-volt rheostat, was wrapped in asbestos to reduce heat loss.

Plexiglas strips, 1-1/16 inch wide by 30 inches long and with a 3/8-inch-deep groove the thickness of the iron bar, were pressed to the sides of the bar. The strips formed a wall along the bar sides; the height of the wall from the top surface of the bar was 7/16 inch. A section of Plexiglas was bonded to both sidewalls near one end of the iron bar, and 25 inches from this section, or end wall, another section of Plexiglas was bonded to form the opposite end wall. These strips enclosed the test area on the heat bar. A thin layer of finely ground cornmeal sifted over the bar served as food. Temperatures within the test area were measured by 15 copper-constantan gasket thermocouples, 0.79 inch in diameter. The thermocouples, spaced about 1 1/2 inch apart, were attached medially along the underside of the bar by means of machine screws.

The entire apparatus was enclosed in a Plexiglas cylinder 35 inches long with a 3 1/2-inch inside diameter. One end of the cylinder was closed with a disk made of Plexiglas. The cylinder, sec-

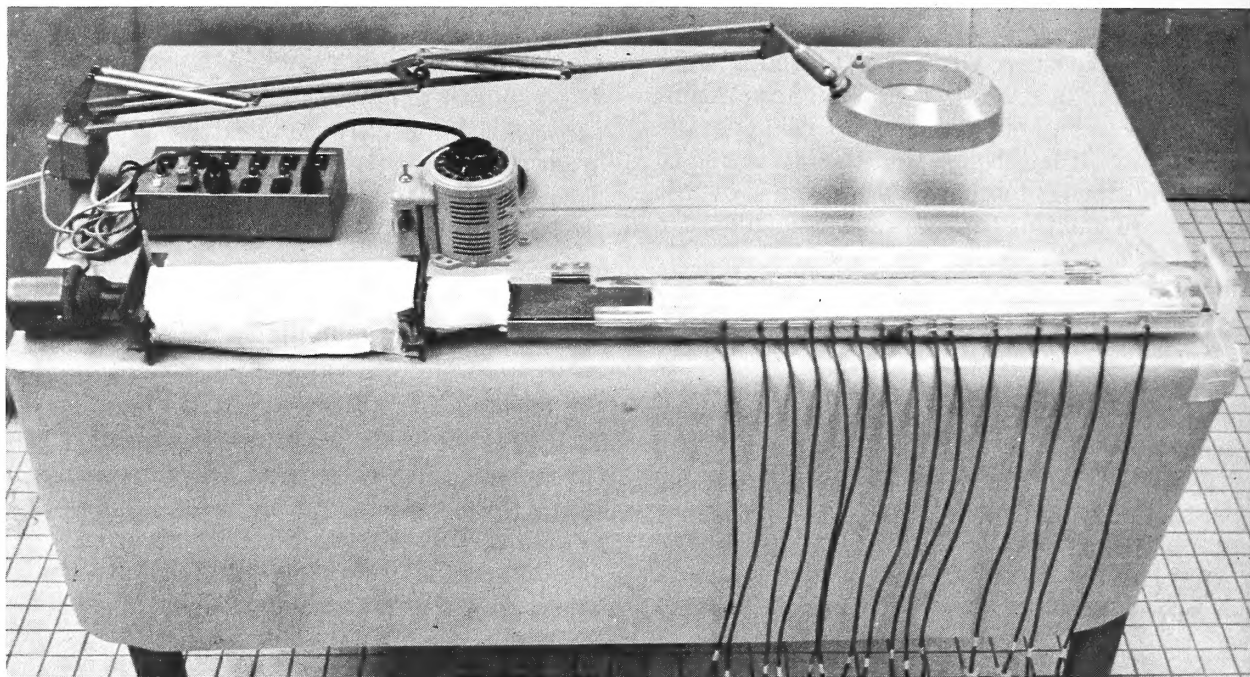


FIGURE 1.—Temperature gradient chamber.

tioned longitudinally, was fitted with two hinges so that the top section could be opened to place and remove larvae. Each temperature gradient chamber was placed and leveled on a table in a 450-cubic-foot room maintained at $57^{\circ}\pm 2^{\circ}$ F and 70% relative humidity. The room was kept dark except during observation.

A temperature gradient from 60° to 90° F was maintained within the test area of the bar. Temperatures were monitored at regular intervals with a multipoint recorder. A humidity gradient dependent on the temperature gradient was measured along the bar immediately above the cornmeal and 1 inch above the bar. Relative humidity readings were made at temperatures of 65° , 80° , and 90° F. Relative humidity measurements above the bar were made by a variable resistor sensor (Hydrodynamics, type TH narrow-range sensors, class A). Readings were made after the sensor, held firmly by two burette clamps mounted on a ring stand, had been inserted and positioned through precut holes made in a temporary plastic top. The temporary cover, constructed of 10-mil polyethylene plastic, was secured to the Plexiglas bottom by masking tape. This cover followed the configuration of the original top. Holes for the sensor were covered with masking tape when they were not be-

ing used. The moisture content of the cornmeal layer on the bar, as related to relative humidity, was determined in the following manner: 2-gram samples were collected from the selected temperature point (three replicates each) and dried in an air-convection oven at 266° F for 1.25 hours; percentage moisture content of the medium was calculated on a wet basis.

Each test was initiated by placing 100 third or fourth instar larvae on the bar at a selected temperature. Releases were made at 68° F and at 2° intervals through 88° F. After 24 hours temperatures were recorded from all thermocouple positions, and the number of larvae in each temperature zone was noted. All releases at the selected temperature points were replicated three times. Thus, 3,300 larvae of each instar were exposed to temperature gradients during the experiment. The validity of data from these tests was confirmed by releasing fourth instar larvae (1,500) in groups of 125 on the bar at selected temperatures of 70° , 75° , 80° , and 85° F. The larvae were exposed on the bar for 168 hours, and then their location on the bar in reference to the temperature gradients was recorded. This test was replicated three times. In addition, fourth instar larvae were released on the bar at 61° F. The distribution of

these larvae (two replicates of 100 larvae each) was recorded after 96 hours.

In all tests larvae were counted manually with the aid of an illuminated magnifier. Larvae and cornmeal were removed after each replicate with an aspirator. Fresh cornmeal and untested larvae were used in each replicate. Test larvae were obtained from our insectary, which is maintained at $80^{\circ} \pm 2^{\circ}$ F and $70\% \pm 2\%$ relative humidity. Larvae were held at 75° F for 1 hour before being placed on the bar.

Results and Discussion

The dispersion of third and fourth instar cigarette beetle larvae in a temperature gradient is shown in figure 2. The preferred temperature range of both instars was 78° to 82° F. It appeared that the initial release point had some influence on dispersion. A sluggish response was readily discernible in the dispersal patterns of larvae released in the cooler areas. However, this response may have been a form of "cold trapping" and was less evident in tests with fourth instar larvae that remained on the bar for 168 hours (fig. 3). While the curve slopes in both 24- and 168-hour tests show a similar dispersion pattern, larval preference for 78° to 82° F was

more clearly demonstrated during the longer test. The response reported for larvae at 97° F actually reflects larval mortality. Larvae entering this higher temperature gradient became static and died.

Although moisture gradients were not controlled during our tests, constant water contents based upon temperature gradient were soon established. Cornmeal placed on the bar with a moisture content of 10.3% gave water content gradient readings of 7.3%, 5.9%, and 4.0% at temperatures of 65° , 80° , and 90° F, respectively, after 24 hours. Correlational relative humidity readings for these water content readings at these temperatures were 23%, 17%, and 8%, respectively. Measured relative humidities (electronic sensor) at these same temperatures immediately above the cornmeal were 28%, 23%, and 20%, respectively. Likewise, 1 inch above the bar, readings were 28%, 25%, and 23%. Thus, a humidity gradient stratification of 17%, 23%, and 25% was present at 80° F, which is within the temperature gradient preferred by the larvae.

We believe that the response of larvae to the 78° – 82° F gradient was mostly due to temperature, but the humidity gradient cannot be en-

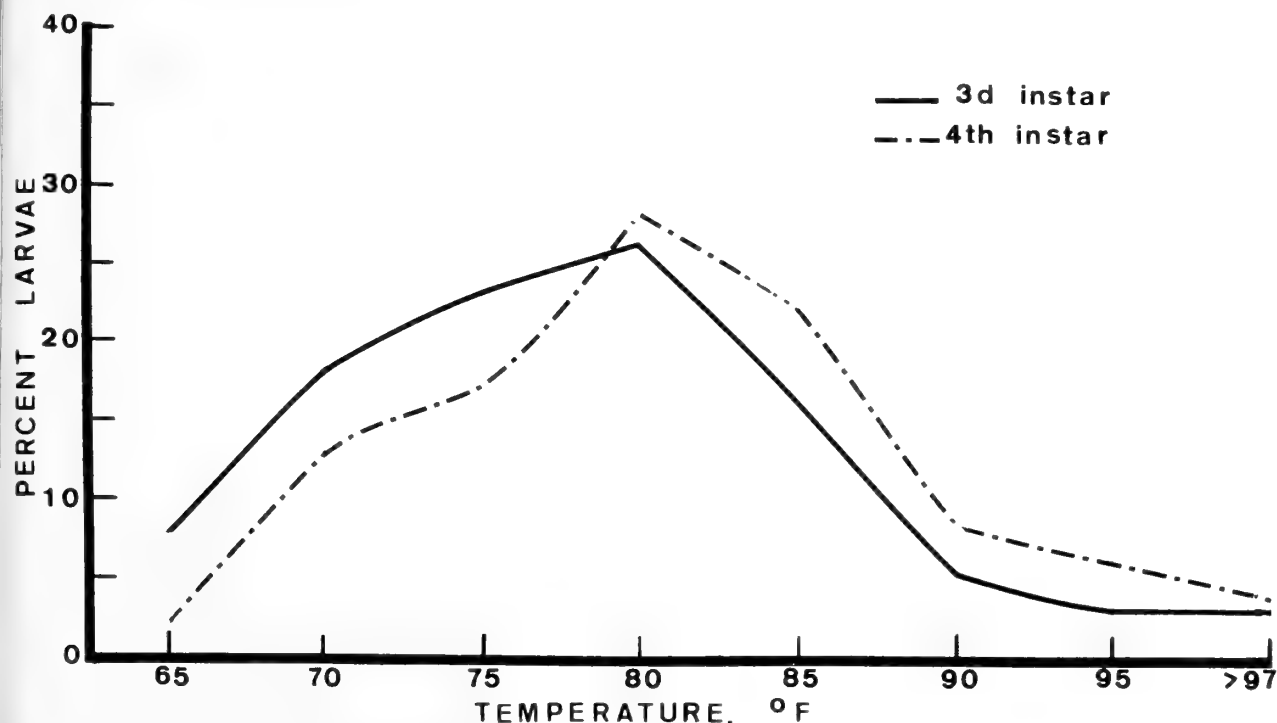


FIGURE 2.—Dispersion of third and fourth instar cigarette beetle larvae in a temperature gradient. All temperature readings $\pm 2^{\circ}$ F.

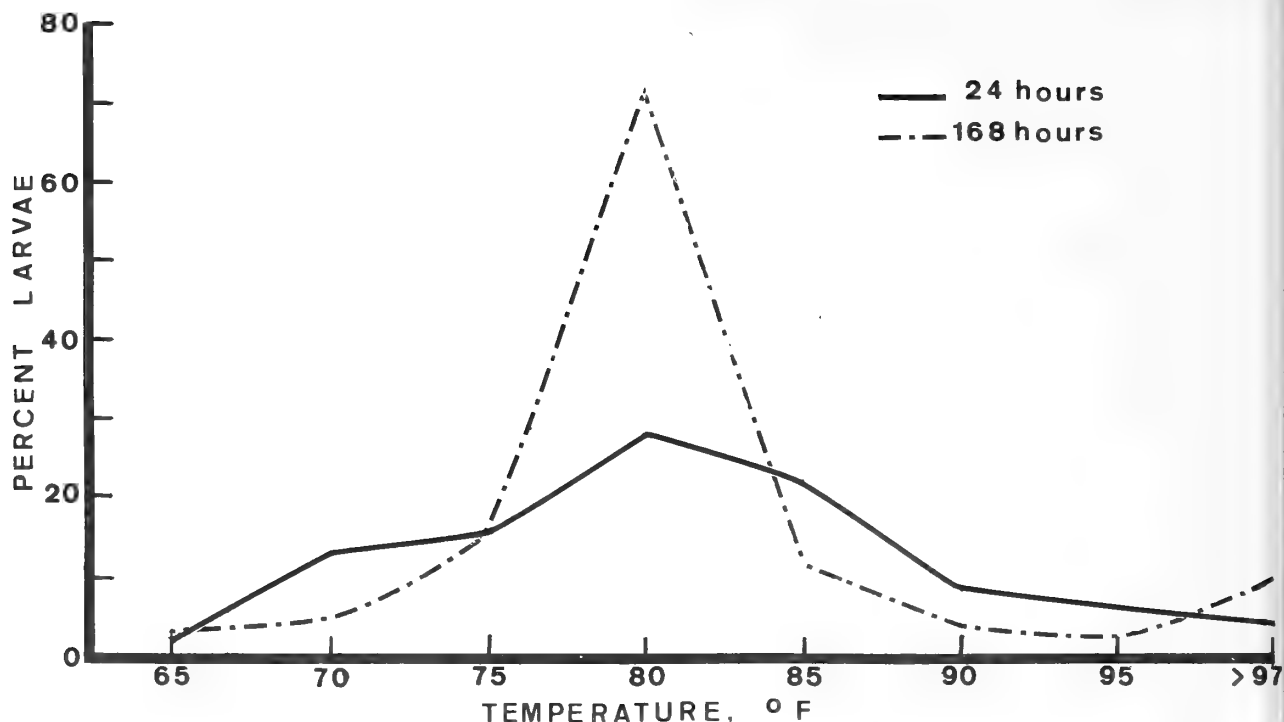


FIGURE 3.—Dispersion of fourth instar cigarette beetle larvae in a temperature gradient after 24 and 168 hours. All temperature readings $\pm 2^{\circ}$ F.

tirely discounted. The hygronegative response of cigarette beetle larvae under constant temperature (section 1) supports the belief that temperature was the main factor dominating the response, because lower humidities were available to the larvae at higher temperatures. It seems advisable to consider the joint action of temperature and humidity on the larvae.

The possibility that other factors apart from temperature may affect behavior in a temperature gradient has been stressed by Madge (11). Among others, Yinon and Shulov (17) consider that insect behavior is markedly affected by preconditioning. The fact that our results from tests

of 24 and 168 hours' duration were similar (varying only in degree of response) encourages us to believe that preconditioning, if it occurred, had no significant effect on overall larval response.

In the test where fourth instar larvae were released at 61° F, 31% moved to temperatures of 70° F or more in 96 hours. Investigations are needed to ascertain the critical low temperature where cigarette beetle larvae become immobile. There have been instances where larvae have dispersed in hogsheads of tobacco at temperatures lower than 61° F under warehouse conditions.

SECTION 3.—LARVAL EMIGRATION FROM TOBACCO HOGSHEADS IN A WAREHOUSE

Materials and Methods

Five hogsheads of tobacco infested with cigarette beetles were maintained in the laboratory for 2 months at 75° F and 30% to 40% relative humidity. Three hogsheads were taken to a tobacco warehouse and placed in a chamber constructed of 20- by 20-mesh fiberglass screen wire

(fig. 4), which prevented the beetles from escaping and infesting other hogsheads in the warehouse. The cases were removed from two of these hogsheads to facilitate observation of conditions occurring on the surface of the tobacco. The two hogsheads remaining in the laboratory were controls.

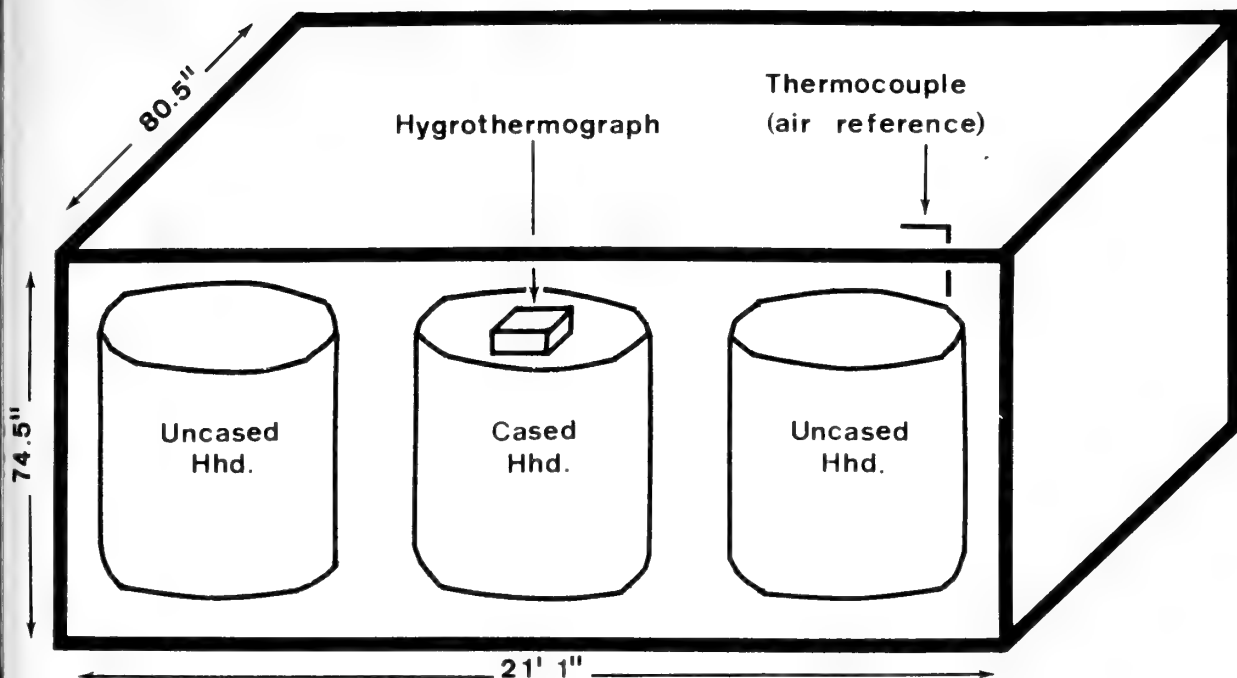


FIGURE 4.—Arrangement of tobacco hogsheads in screened chamber located in tobacco warehouse.

The placement of the sensing elements in the cased hogshead is shown in figure 5. Copper-constantan probe thermocouples were installed in the hogsheads for temperature measurement on the surface and at depths of 2, 4, 6, 12, and 24 inches. Temperatures were recorded by a multipoint recorder activated by a time clock every 2 hours. Variable resistor sensors were used to measure relative humidity. Each of three sensing elements were fitted with a Tygon collar so that

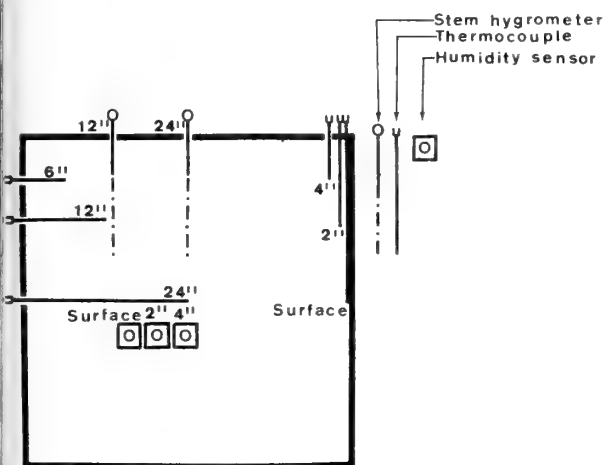


FIGURE 5.—Sensing elements in cased hogshead of tobacco (side view). Dimensions in inches indicate depths in tobacco at which readings were taken.

a reading could be taken only from the uncovered end. One sensor was installed on the surface, and the other two were placed at depths of 2 and 4 inches in the tobacco. Relative humidity was monitored continuously by a three-channel recorder. Additionally, stem hygrometers (18 inches long) with synthetic hair elements (Lambrecht) were placed in the tobacco at 12- and 24-inch depths to measure relative humidity. Readings from these were taken daily at 8:00 a.m. The uncased hogsheads were monitored in a similar manner except that variable resistor sensors were not present.

A hygrothermograph placed on the cased hogshead (fig. 4) measured the temperature and relative humidity of the air space. Air space temperatures were also monitored by a thermocouple placed at the top of the screened chamber. All instruments were calibrated each month so that the accuracy of each was within $\pm 1^\circ \text{F}$ and within $\pm 3\%$ relative humidity. The floor space surrounding the hogsheads was covered with paper and examined daily to determine if larval emigration had occurred.

Results and Discussion

Cigarette beetle infestations in prized tobacco are usually within 2 inches of the perimeter of the hogshead. For this reason, a detailed analysis

of the conditions occurring in this area was made.

The average daily temperatures and relative humidities at the surface of the tobacco hogsheads and a depth of 2 inches for 28 days are shown in figure 6. Larvae crawled out of the hogsheads and dropped to the floor sometime during the period between 8:00 a.m. of the 20th day and 8:00 a.m. of the 22d day. Larval emigration occurred again on the 27th day. The average temperature at a depth of 2 inches was never below 55° F before the 18th day. The temperature changes at the surface and at a depth of 2 inches within the tobacco for a period of 10 days during which the two emigrations occurred are shown in greater detail in figure 7. The surface temperature was below 55° F for 32 hours, from 2:00 p.m. of the 18th day to 10:00 p.m. of the 19th day. Temperatures at the 2-inch depth in the tobacco were below 55° F for 28 hours, from 10 p.m. on the 18th day to 2 a.m. on the 20th day. This established a gradient of cooler to warmer temperatures in the first 2-inch depth in the tobacco. Beginning at 10 a.m. on the 20th day, the surface temperature was above 60° F for 56 hours; for 36 of those hours it was above 65° F, and for 12 hours the temperature was above 70° F. The temperature at the 2-inch depth

was above 60° F for 56 hours also, but was only above 65° F for 28 hours and never above 70° F. Relative humidities at the surface of the tobacco were lower than were the relative humidities at the 2-inch depth for the first time during this warming trend (fig. 8). The larvae were seen leaving the hogshead during the warming period.

Conditions occurring before larval emigration on the 27th day of the test were as follows: For a period of 84 hours from 10 p.m. on the 22d day to 10 a.m. on the 26th day, the tobacco surface temperature was below 55° F. The temperature at the 2-inch depth in the tobacco was below 55° F for 80 hours beginning at 2 a.m. on the 26th day. This was followed by a warming trend in which the surface temperature was above 60° F for 20 hours, above 65° F for 12 of the 20 hours, and above 70° F for 4 of the 20 hours. The temperatures at 2 inches in the hogsheads were above 60° F for 24 hours, but only above 65° F for 8 hours and never above 70° F. The relative humidities at the surface and at 2 inches in the tobacco were the same during this period. The total number of larvae leaving the tobacco during the two emigrations exceeded 1,200, and all were the fourth instar. No larvae emigrated from the controls during this period.

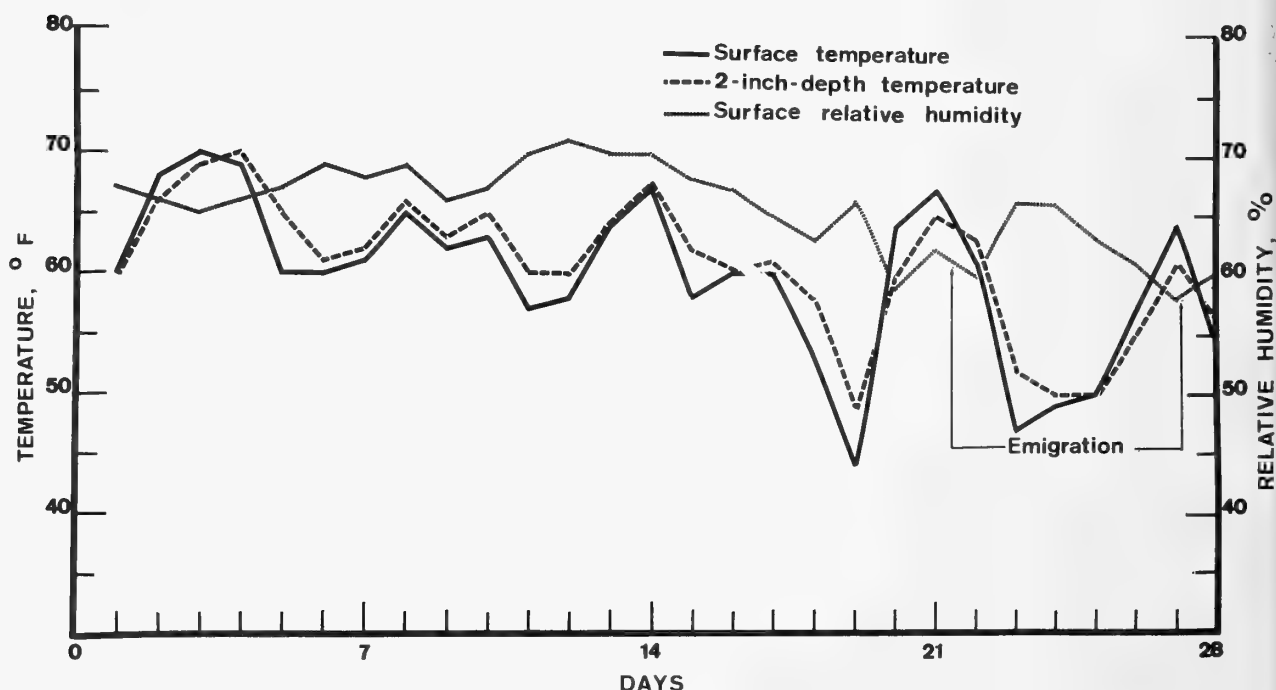


FIGURE 6.—Average daily temperatures and humidities recorded before and after two larval cigarette beetle emigrations.

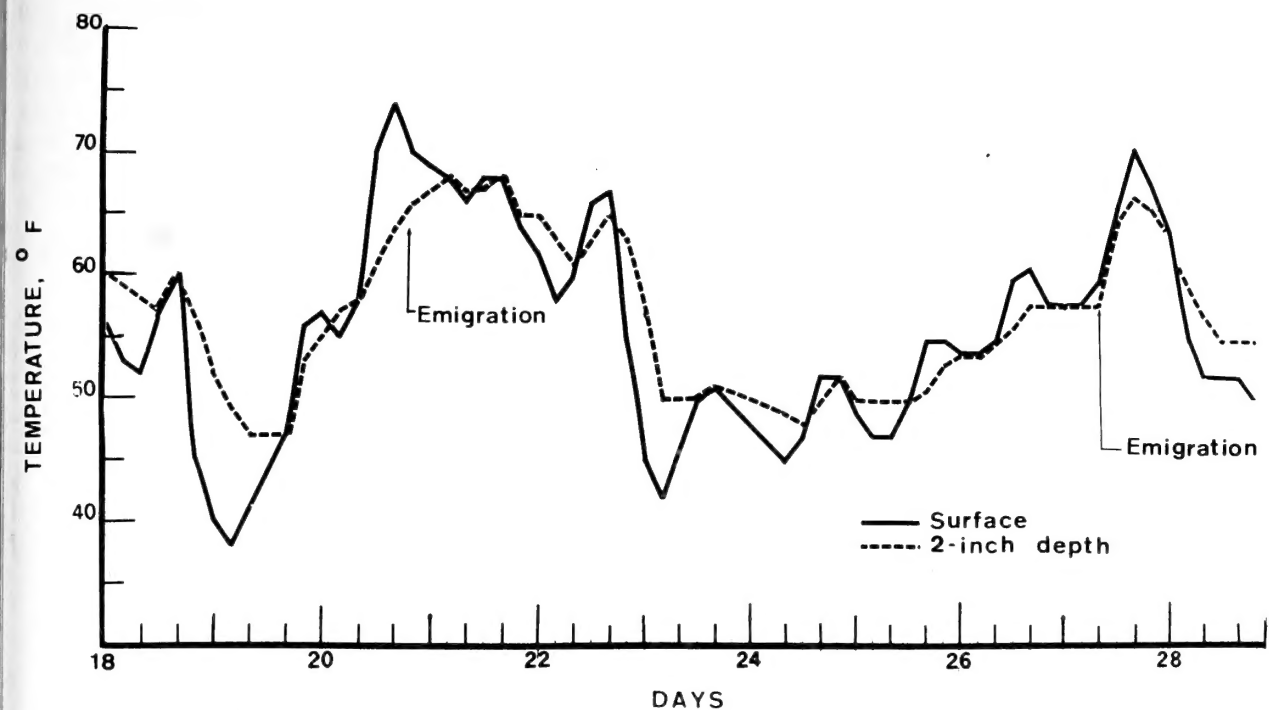


FIGURE 7.—Average 8-hour temperatures at the surface of a hogshead and at a depth of 2 inches in tobacco during the 10-day period in which two cigarette beetle emigrations occurred.

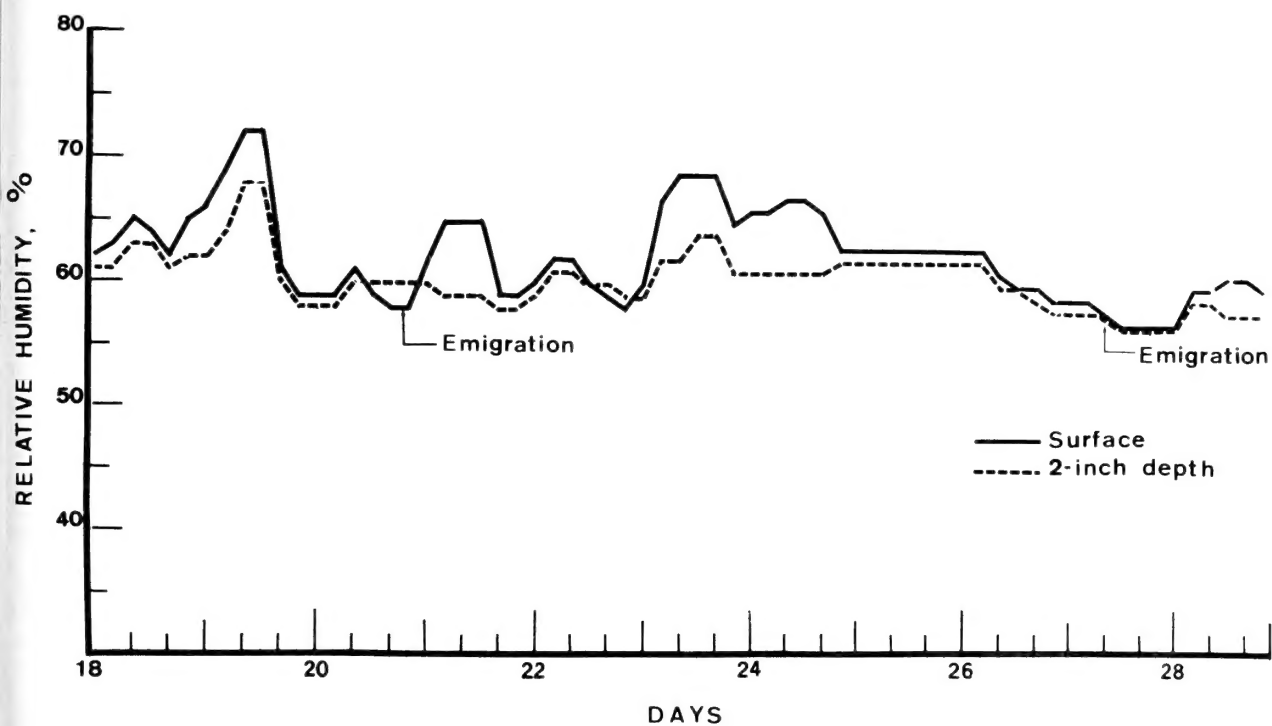


FIGURE 8.—Average 8-hour relative humidities at the surface and 2 inches in tobacco during the 10-day period in which two cigarette beetle emigrations occurred.

We believe that a cooling period of sufficient length followed by a warming weather cycle triggered larval movement. Under these conditions, larvae emigrated to the surface of the tobacco, where it was warmer, and fell to the floor. As no suitable overwintering habitat was available, the larvae eventually died from cold. It appears that

temperature may play a more significant role in triggering larval movement from tobacco hogsheads than does relative humidity. However, further study is necessary, since there was a decrease in relative humidity during the warming cycle, which might have intensified the larval movement toward a warmer area.

CONCLUSIONS

Cigarette beetle larvae invariably select the lower humidity when presented with two or more alternatives and move toward a preferred temperature zone (78° to 82° F). The fact that cigarette beetle movement can be predicted in controlled temperature and humidity gradients may be of practical use. However, the phenomenon of larval cigarette beetle emigrations from stored tobacco under warehouse conditions needs

further study. The response of larvae to temperature and humidity gradients indicates that it may be possible to manipulate the environment so that emigration would be assured. An economical method would have to be devised to establish the proper temperature and humidity gradients before larval emigration could be used to control the cigarette beetle in hogsheads of stored tobacco.

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